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TITLE: EVALUATION OF NEW ANTIMALARIALS

SUBTITLE: Development of New Antimalarial Drugs

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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CDRI-WRAIR MALARIA PROJECT

(1989-1990)

"DEVELOPMENT OF NEW ANTIMALARIAL DRUGS"

Objectives of the project:

I. (a) To screen new potential antimalarial compounds originating from Walter Reed Army Institute of Research (Washington) at the Central Drug Research Institute, Lucknow.

(b) To maintain in operational state the existing antimalarial test systems established at CDRI for blood schizontocidal, radical curative (anti-relapse activity) and causal prophylactic efficacy tests against P.cynomolgi B and to introduce new simian screening model for gametocytocidal/sporontocidal efficacy.

(c) To ensure that base-data and dose revalidation with standard drugs, namely chloroquine and primaquine to be used as reference compounds, are reproducible.

(d) To screen potential compounds for causal prophylactic activity against P.cynomolgi and detailed preclinical evaluation of selected compound.

(e) To establish single, two or three dose bioassay for selected promising antimalarial compounds for their efficacy against blood/tissue stages.

II. To take stock of the research accomplishments/major achievements in the area of development of new tissue schizontocides under previous CDRI-WRAIR collaborative project No. DAMD

-17-82-G-9515 (1982-1988) and to develop joint research programme for further pre-clinical development of most active/new lead compounds.

III. (a) To establish capability for in vitro screening of potential compounds and natural products available at CDRI for antimalarial efficacy against P.falciparum and to identify new promising candidate compounds for control of drug resistant falciparum malaria which is fast spreading.

(b) Train manpower in large-scale in vitro antimalarial screening programme and transfer of this Technology from WRAIR to CDRI.

Background

The resurgence of malaria in most of the tropical countries including India in recent years has posed a serious health problem since the disease causes a good deal of morbidity and mortality particularly in rural areas. The malaria situation has become alarming because of three reasons: Firstly, some strains of P.falciparum resistant to the conventional antimalarial drugs are gaining ground in South-East Asia and have already made their presence felt in the North-Eastern part of the country. Some of these falciparum cases occasionally develop cerebral complications. Secondly,the choice of tissue schizontocides for use in vivax malaria cases, is limited to only primaquine which is toxic and has to be given over a prolonged period and can not ordinarily be used in children and pregnant women. Thirdly there is no effective drug for prophylaxis against vivax malaria which constitutes 85% of the malaria cases. It is thus obvious that there is an urgent need for the development of safe causal prophylactic agents as well

as new and safe tissue schizontocides for preventing relapse of vivax malaria and of quick acting blood schizontocides against drug-resistant falciparum cases and for the treatment of cerebral malaria cases.

The collaborative project (1982-88) between Walter Reed Army Institute of Research, Washington and Central Drug Research Institute, Lucknow has given a boost to the global efforts to develop new drugs for control of malaria. Two candidate tissue schizontocidal drugs have been identified, which are relatively safer than primaquine. Both these compounds are proposed to undergo phase I clinical trials and subsequent efficacy tests as anti-relapse drugs. The leads generted from the programme has given new dimensions to our programme for synthesis of potential antimalarial compounds. The technology including test systems established at CDRI through the sustained efforts and collaboration between U.S. and Indian scientists over the years, would go a long way to devleop new future drugs for control of malaria.

3. MAINTENANCE OF OPERATIONAL ANTIMALARIAL SCREENS FOR PRIMATE MALARIA

I. The following antimalarial screens developed at CDRI under the CDRI-WRAIR project DAMD-17-82-G-9515 (1982-1987) have been maintained in operational state for ongoing collaborative programme between CDRI-WRAIR.

(a) Rhesus blood schizontocidal test.

(b) Rhesus radical curative (anti-relapse) test.

(c) Rhesus prophylactic test.

(d) Protocol for cyclic passage of P.cynomolgi B.

II. Protocol for development of new gametocytocidal/sporontocidal agents have been established using P.cynomolgi B - A.stephensi model, and the identified compounds will be evaluated for efficacy against P.falciparum gametocytes. This programme will eventially help to control transmission of drug-resistant malaria.

PROTOCOLS DEVELOPED AT CENTRAL DRUG RESEARCH INSTITUTE, LUCKNOW IN COLLABORATION WITH WALTER REED ARMY INSTITUTE OF RESEARCH FOR DEVELOPMENT OF NEW ANTIMALARIAL DRUGS

During the last nine years, CDRI, Lucknow, has been working in close collaboration with WRAIR, Washington and AFRIMS, Bangkok for standardization of experimental models for screening of new blood schizontocidal, radical curative (anti-relapse) and causal prophylactic agents and the pre-clinical efficacy trials of the compounds synthesized and developed by US Army antimalarial drug development programme and the CDRI. This joint biomedical collaborative research programme has led to the establishment of reliable antimalarial screening models. The reproducibility of the following antimalarial screens at CDRI has been validated using standard drugs and it is encouraging to point out that our resuls with the standard drugs are in close agreement with the data obtained at AFRIMS, Bangkok and WRAIR, Washington. The test systems are in operation and it is proposed to keep these antimalarial models operational for ongoing collaborative programme between CDRI and WRAIR.

A. RHESUS BLOOD SCHIZONTOCIDAL TEST:

(a) Maintenance of rhesus monkeys (M.mulatta):

Rhesus monkeys (4-5kg) used for antimalarial screening programme are procured from approved Government Contractors, and kept under quarantine for four weeks. These monkeys are tuberculin tested before receiving them in the primate house and then after every 1 to 2 months. During quarantine period monkeys are

chest X-rayed and examined for absence of blood protozoans and three blood smears at weekly intervals are preserved for records. Tuberculin negative monkeys free from any blood parasites are transferred to experimental wing and kept in mosquito free rooms. They are supplied standard pellet diet, seasonal fruits, vegetables and water ad libitum. The monkeys are kept under 12 hrs. photoperiodicity.

(b) Parasite:

Plasmodium cynomolgi bastianelli (B strain) procured in 1979 from Dr. W.E.Collins, CDC, Atlanta, has been maintained in CDRI by successive blood induced passages as well as by cryopreservation. During the current WRAIR-CDRI collaborative project, the parasite P.cynomolgi B has been transmitted, since Nov. 1982, through Anopheles stephensi for 72 consecutive passages and the parasite taken from patent infection has been used from time to time for standardization of blood schizontocidal test using chloroquine diphosphate as the reference drug.

For blood induced infections, the rhesus monkeys are infected with $1x10^5$ parasitized RBC in 1 ml. of acid-citrate dextrose (ACD) intravenously and blood smears are examined daily for patency. The parasitaemia is recorded in terms of number of parasities/mm^3 from thick or thin blood smears. At the patency, the parasitaemia is recorded in thick smears on the basis of number of parasites per 50 oil immersion fields. The parasite number multiplied by 20 gives parasitaemia/mm^3. When number of parasites/50 thick fields is more than 50, further recording is made from thin films by determining the number of parasites/100 WBC and parasitaemia/mm^3 is cacculated after recording number of WBC/mm^3 using haemocytometer Finally when number of parasites/100 WBC is more than 1000, further

recording is made by counting the parasitized cells/10^4 RBC and parasitaemia/mm^3 is calculated after determining the number of RBC/mm^3 using haemocytometer.

(c) Staining of blood films:

Improved method of recording parasitaemia used at AFRIMS has been introduced in the project, using thick/thin blood smears which are subsequently stained with Giemsa stain.

Giemsa (liquid) BDH/(Glaxo)	= 5 ml.
Acetone (A.B)	= 5 ml.
Phosphate buffer M/15	= 90 ml.
(pH 7.2)	

Both thick and thin films are made on the same slide. The thick film is prepared from 2 drops of ear-vein puncture blood and spread approximately in a 10 mm area and dried in front of air blower. The thin blood film is fixed in methanol for 1 minute and slides are put in diluted Giemsa stain. Slides are stained for 40-45 minutes and parasitaemia is recorded as described above.

(d) Drug administration:

When asexual parasitaemia reaches above 5000/mm^3, drug treatment is initiated. The drug is suspended in distilled water or 0.3% methyl cellulose solution and daily dose is administered orally in 10 ml. via a catheter, followed by 5 ml. water for flushing the catheter. The drug is preferably administered in morning before meals and monkeys are observed for 20-30 minutes for any vomitting sensation.

(e) Determination of chloroquine curative dose:

(1.0 mg chloroquine base = 1.62 mg chloroquine diphosphate)

In order to determine 100% curative dose of chloroquine against blood induced P.cynomolgi B in rhesus monkey, ... initial

infective inoculum was taken from monkey infected by sporozoites. For conducting the blood schizontocidal test, five groups of monkeys each were given $1x10^5$ parasitized RBC by i/v route and when the parasitaemia had reached 0.1 to 0.5% level (5,000-20,000/mm^3), each group (5 monkeys) was administered chloroquine dosages of 1.0, 3.0, 5.0, 7.0 and 10.0 mg/kg (base) for 7 days by oral route. Both thick and thin smears were examined daily to monitor the course of parasitaemia. Our results show that all the 5 monkeys at 1mg/kg dose showed recrudescnece, while those at 3.0, 5.0, 7.0 and 10.0 mg/ml were cured radically and showed no recrudescence till day 30. The 5 monkeys showing recrudescence of parasitaemia at 1.0 mg/kg were retreated with the next higher dose i.e. 3.0mg/kg x 7 days. This dose was again found to be curative and no recrudescence was observed till 30 days.

Revalidation of curative dose of chloroquine. Three batches of 5 monkeys were given blood induced infection from a sporozoite infected monkey. When the parasitaemia had reached 0.1 to 0.5% level, they were administered chloroquine at 3.0, 5.0 and 7.0 mg/kg (base) x 7 days by oral route, and all the monkeys were followed for 30 days. All the three doses were again found to be curative.

Both initial curative tests and revalidation tests thus show that 3.0 mg/kg x 7 doses of chloroquine (base) have curative action on blood induced P.cynomolgi B. Although 3.0 mg/kg chloroquine has been found to be consistantly curative, we prefer to use 5 mg/kg chloroquine as the curative dose of radical curative tests. This dose we have used in radical curative tests and we have

found it satisfactory.

The dose validation was again carried out using parasites obtained from infected monkey in the XXXVth serial sporozoite passage. Three monkeys each were treated with chloroquine at 1.0, 3.0, and 5.0 mg (base)/kg dose levels. Monkeys treated at lowest dose of 1.0 mg/kg showed recrudescence on days 11, 17 and 25, while monkeys at higher dose levels were protected.

B. RHESUS RADICAL CURATIVE (ANTI-RELAPSE) TEST (SEVEN DAY SCHEDULE:

Simian malaria parasite p.cynomolgi B, which closely resembles human malaria P.vivax in its biological characteristics and relapse patterns, has been used for anti-relapse efficacy tests using 7 day treatment radical curative test in sporozoite induced infections.

Primaquine has been used as the reference anti-relapse drug and chloroquine has been invariably used as the companion blood schizontocide. Both the drugs were administered orally. Although chloroquine is known to have no efficacy against tissue stages of p.cynomolgi which causes relapse of blood parasitaemia, we have to use chloroquine as a companion blood schizontocide in curative doses to effectively eliminate all blood parasitaemia from patent monkeys. From the day of sporozoite inoculation upto day 8; the primary tissue stages of P.cynomolgi B develop in the hepatocytes and after completion of phase of primary exo-erythrocytic cycle, the parasite invade blood and infecte red blood cells. Generally the monkeys become patent (i.e. slide positive blood smears) on day 8-12 depending upon the sporozoite inoculum. Once the monkey becomes patent, as shown by blood smear examinat-

-ion, there is 100% evidence of establishment of sporozoite induced malaria infection in the rhesus monkey. In order to study the effect of primaquine and related compounds on secondary tissue stages (Hypnozoites; which cause relapse), we have to administer a otally curative dose of chloroquine to eradicate blood infection. A total curative dose of chloroquine would ensure elimination of all asexual erythrocytic stages and if the chloroquine dosing is inadequate, it would lead to recrudescence of parasitaemia, thus interfering with the interpretation of radical curative efficacy of test compound. Any patent infection after curative chloroquine treatment would be interpreted as relapse due to failure of the anti-relapse test compound.

Determination of primaquine curative dose:

(mx primaquine base = 1.76 mg primaquine diphosphate)

For the determination of the radical curative dose of primaquine 2 monkeys each were treated orally at 0.180, 0.316, 0.568, 0.730, 1.0, 1.3, 1.795 and 5.680 mg (base)/kg dose levels. The treatment was initiated when blood parasitaemia reached 5000/mm^3 and administered once daily for seven days. The blood smears were examined for 90 days after end of treatment for appearance of relapse infection. The results shwed that monkeys treated at 0.180 mg/kg and 0.316 mg/kg relapsed while monkeys treated at higher doses were cured. The relapsing monkeys were re-treated at 1.0 mg (base)/kg dose x 7 days and these monkeys were cured.

For the revalidation of radical curative dose, the treatment was given orally at 1.0 mg/kg dose to 8 monkeys and 1.3 mg/kg to 5 monkeys. All the 13 treated monkeys were cured of infection.

. The dose of 1.0 mg/kg (base) is being used as the standard radical curative dose in our study. This dose of primaquine is combined with 5 mg/kg (base) chloroquine as the curative blood

schizontocidal drug.

Primaquine at 1.00 mg/kg (base) x 7 days was also found to be curative in 3 monkeys after intravenous administration (Table-6). Although initial reports from Walter Reed indicated escalation of radical curative primaquine dose and chloroquine curative dose over the years at AFRIMS, the dose standardization carried out at CDRI has been much more consistent in successive cyclic passages during the last 9 years and no escalation of primaquine/chloroquine curative doses has been observed at CDRI (Table-I).

C. RHESUS PROPHYLACTIC TEST (Three day schedule):

The methodology for initiating sporozoite induced infections with P.cynomolgi B in rhesus monkey has been described later and the same has been applied in the prophylactic test. A three day treatment model has been standardized for evaluation of potential causal prophylactic compounds. In this model, the drug treatment is administered on day -1, 0 and +1 and sporozoite infection is inoculated on day 0. The blood smears are examined from day 7 onwards till day 70 for observing the patency in experimental animals. This model has advantage over conventional 9 day treatment model developed by Schmidt, since the treatment schedule is for shorter duration.

(a) Drug Administration:

The test durg is administered for three doses (on day -1, day 0, day +1 of sporozoite inoculation) orally in 10 ml. volume. The drug/test compound is suspended in 0.3% methyl cellulose solution.

(b) Determination of primaquine prophylactic dose:

For determining the prophylactic dose of primaquine, experimental monkeys were treated at 0.316 mg/kg, 1.00 mg/kg, 1.78

mg/kg, 3.16 mg/kg and 10.00 mg/kg x 3 day, dose levels. Our results showed that monkeys treated at 0.316 mg/kg, and 1.00 mg/kg became patent while higher doses of 1.78 mg/kg and above were curative (Table-2). The curative dose of 1.78 mg/kg has been revalidated several times during the 44 successive cyclic passages of P.cynomolgi B and this dose is being used as the standard reference dose in our study (Table-3). This test system was not operational at WRAIR/AFRIMS and has been standardized at CDRI.

In the 9 day schedule prophylactic test, where primaquine was administered on day -1, 0 and +1 to +7, dose of 1.0 mg/kg base was curative in 5/6 monkeys. Treatment with higher dose at 2.0 mg/kg protected 4/4 monkeys.

D. PROTOCOL FOR CYCLIC PASSAGE OF P.CYNOMOLGI B;

P.cynomolgi B in monkey attains primary peak parasitaemia of 4,00,000 - 8,00, 000/mmm^3 in 7-8 days after patency. The parasite number then declines without any treatment and the secondary peak parasitaemia of 30,000-60,000/mm^3 is observed 4-5 days later. Initial studies at AFRIMS have shown that maximum infectivity of mosquitoes is observed when they are fed on infected monkeys during the secondary peak parasitaemia. Moreover, a ratio of 3:1 for female to male gametocytes has been found to be ideal for obtaining maximum infectivity. In order to ensure high infectivity of mosquitoes, the above practice is followed.

(a) Insectary:

In order to develop technology for large-scale sporozoite production for rhesus monkey inoculation, a large scale rearing of Anopheles stephensi (NICD Strain) for transmission studies had been set up. The insectary maintains the availability of all the four larval instars at all times and has an average production

of 3000-6000 pupae daily. Rhesus monkey has been found to be ideal for giving blood meal to mosquitoes for egg laying. The larval stages are fed on powdered yeast and maintained at 25±1°C. The pupation starts after 8-10 days and adults emerge 36 to 48 hr. later. The adults are maintained at 26±1°C with relative humidity 75 to 80%, and fed on 5% multivitamin solution.

(b) Infection of mosquitoes:

Two or three days old female mosquitoes (Anopheles stephensi) are fed on infected rhesus monkeys showing optimum gametocyte number/ratio. The monkey is anaesthetized with sodium intraval (20mg/kg i/v) and mosquitoes are allowed to have blood meal for 20-30 minutes. Three batches of mosquitoes are fed on each monkey on three consecutive days. The fed mosquitoes are kept in insectary whereas the males and unfed females from each cage are discarded.

On day 7 after blood meal, 5 mosquitoes from each of the infected batch are dissected for determining the number of oocyst on the gut. The mean number and the size of the oocysts is recorded, which generally ranges between 20-60.

(c) Estimation of sporozoite number of infected mosquitoes:

On day 13 after blood meal (or one day prior to inoculation of monkeys), the sporozoite number is estimated in the mosquitoes from the batch which has earlier been found to show high oocyst number on day 7. The infected mosquitoes from the sample batch are anaesthetized and legs, wings, head and abdomen are removed. The thoraces are grinded with a mixture of 0.5 ml saline and 0.5 ml rhesus normal serum (Total 1 ml.). With the help of a graduated capillary (or an eppendorf pipette), 5 µl of the sporozoite suspension is applied and spread within the

etched circle of a FA slide. The slide is allowed to air dry, fixed in methanol (5 minutes) and stained with Giemsa stain for 40 minutes. The number of sporozoite/100 oil immersion fields is counted in two slides and the mean vlaue multiplied by the magnification factor of the microscope gives the sporozoite number per ml. of solution or the sporozoite number/10 infected mosquitoes. (The magnification factor for our microscope as determined by using a slide/stage micrometer is 10974). From this estimation, the number of mosquitoes required to obtain 1 million sporozoites/ monkey can be determined.

(d) Harvesting of sporozoite for inoculation:

On day 14, sporozoites are harvested from the infected mosquitoes for inoculation into rhesus monkeys. Required number of mosquitoes (determined on the basis of estimation made on day 13) are anaesthetized by keeping them for 2-3 minutes in a referigerator and their legs and wings are removed. For all further processes the mosquitoes are kept over ice to maintain the infectivity of sporozoites. The head and abdomen of the mosquitoes are removed with a scalpel and thoraces are put in a chilled pestle-mortar for grinding. The grinding is carried out in cold using 1:1 mixture of normal saline and normal rhesus serum. After grinding, the suspension is centrifuged at 1000 rpm for 15 seconds at 4°C. The sediment is discarded and the supernatant is diluted with serum/saline mixture to get the required inoculum. (Nearly 50% of the sporozoites are lost with the debries after centrifugation). The whole process after anaesthetization of mosquitoes to the inoculation of monkeys should be completed within 45-60 minutes. Each monkey is inoculated via i/v route with 1 ml. of the inoculum, and the exact number of sporozoites inoculated per monkey is determined from the sample inoculum.

(e) Serial cyclic passage of sporozoite induced infection in rhesus monkey:

The recording of parasitaemia in first to fifth passage was made using thin blood films for recording patency. After 5th passage the parasites were kept frozen for nearly 4 months. The gametocytes of the monkey infected with frozen blood were used for infection and for initiation of the 6th passage. In subsequent passages, the patency was recorded from thick smears. Improved method of sporozoite count was also introduced at this stage following the training of Dr. S.K.Puri at AFRIMS. To date the strain of P.cynomolgi B has been cyclically transmitted through A.stephensi for 65 passages .

DEVELOPMENT OF NEW CAUSAL PROPHYLACTIC ANTIMALARIALS:

The present Walter Reed - CDRI Contract proposed to devleop new causal prophylactic agents. Primaquine which is the only prophylactic drug effective against both falciparum and vivax malaria produces several adverse side effects including methemoglobinemia, hemolytic anemia in G-6-PD deficient cases, gastrointestinal disorders and is contraindicated for use in infants and pregnant women. In order to protect healthy population in endemic areas against malaria it is important to develop safe and effective new causal prophylactic drugs. The development of prophylaxis against malaria is becoming all the more important in view of expanding foci of chloroquine and multiple drug-resistant strains of falciparum malaria.

A REVALIDATION OF PRIMAQUINE - PROPHYLACTIC DOSE:

(a) The test model (3-day schedule) for causal prophylaxis using P.cynomolgi B in rhesus monkey is operational at this institute and details of infection of A.stephensi with P.cynomolgi B, harvesting and quantitation of sporozoites for monkey inoculation have been described earlier.

(b) Previous studies with reference drug primaquine for curative dose determination in 3 day treatment schedule have shown that efficacy tests at dose levels ranging between 0.316 mg/kg and 10.00 mg/kg have consistently produced curative action at 1.78mg/kg and higher doses (Table-1). This dose has been revalidated several times against sporozoites obtained from diferent cyclic passages (Table-2).

(c) The primaquine prophylactic dose was again revalidated in Sr. Passage No. 65 during present assignment. Three monkeys each were treated with dose of 1.00mg base/kg, 1.78mg/kg and 3.16 mg/kg for 3 doses on days -1, 0, and +1 of sporozoite inoculation (Table-3). Two of the 3 monkeys treated with 1.00mg/kg became patent on day 36 while the third monkey did not develop patency. Like-wise all the 3 monkeys at 1.78mg/kg as well as at 3.16mg/kg also did not develop patent infection during the observation period of 70 days and were recorded as cured. The untreated control mokey became patent on day 8. The results thus show that there has been no escalation of the primaquine prophylactic dose during the last 8 years. When the paraiste was maintained by serial cyclic passage.

SCREENING OF NEW SYNTHETIC COMPOUNDS

Six new synthetic compounds belonging to the 8-aminoquinoline class have been evaluated for prophylactic activity against P.cynomolgi B using 3 day regimen schedule (drug was administered on day -1, 0, +1).

1. WR 268658:

The compound was tested in two monkeys each at four dose levels viz. 0.0316mg/kg, 0.10mg/kg, 0.316mg/kg and 1.00mg/kg (Table 4). Both the monkeys at 0.0316mg/kg became patent on day 8, two monkeys at 0.1mg/kg were patent on day 10, 11 while two monkeys at 0.316mg/kg were patent on day 14 and 23. Two monkeys at 1.00mg/kg did not develop any patent infection during the observation period (Primaquine index =1.78).

2. WR 268448:

The compound was tested at 0.1mg/kg, 0.316mg/kg and

1.00mg/kg dose levels (Table-5). Both monkeys at 0.10mg/kg became patent on day 10, while two monkeys at 0.316mg/kg developed patency on day 13 and 23. Dose of 1.00mg/kg was curative in two monkeys. (Primaquine index =1.78).

3. **WR 268441**:

The compound was evaluated in two monkeys each at 0.10, 0.316 and 1.00mg/kg dose levels (Table 6). Two monkeys at 0.10mg/kg were patent on day 12 and 17 and one monkey out of two animals treated at 0.316mg/kg developed patency on day 29. The other monkey at 0.316mg/kg as well as both the monkeys at higher dose of 1.00mg/kg were cured. (Primaquine index =1.78).

4. **WR 268648**:

The compound was tested at 0.10mg/kg, 0.316mg/kg and 1.00mg/kg in two monkeys each (Table-7). Doses of 0.1mg/kg (patency day 12, 14) and 0.316mg/kg (patency day 21, 26) were inactive while both the monkeys at 1.00mg/kg were cured. (Primaquine index =1.78).

5. **WR 268404**:

The compound was evaluated at three dose levels in six monkeys (Table 8). Two monkeys at lowest dose of 0.1mg/kg became patent on day 12 and 15. The higher doses of 0.316mg/kg and 1.00mg/kg were curative (Primaquine index =5.63).

6. **WR 268499**:

The compound was tested at 0.10mg/kg, 0.316mg/kg and 1.00mg/kg doses in two monkeys each (Table-9). Dose of 0.10mg/kg was inactive as monkeys became patent on days 15 and 22. While 0.316mg/kg dose was partially curative as one monkey was protected and the other became patent on day 26. The

higher dose of 1.00mg/kg was curative in both the monkeys (Primaquine index =1.78).

CYCLIC PASSAGE OF PLASMODIUM CYNOMOLGI B:

The strain of P.cynomolgi B is being maintained at CDRI viz serial monkey-mosquito-monkey passage since 1982 and to date the parasite has undergone 72 serial cyclic passages. During the period of present report the parasite was maintained through serial passages 63-72 (Table-10). in 12 rhesus monkeys. The patency was recorded on day 8 in 9 out of 12 monkeys and on day 9 in another 2 monkeys. One monkey which was inoculated with lower sporozoite inoculum (1.48×10^5 sporozoites) in serial passage 64 developed patency on day 10. The mean duration for each passage during the present study was 44.89±8.16 days (range 36-59 days).

DIFFERENTIAL SENSITIVITY OF THE PRE-ERYTHROCYTIC STAGES OF PLASMODIUM CYNOMOLGI B TO THE PROPHYLACTIC ACTION OF PRIMAQUINE

The present study was designed to assess the sensitivity of pre-erythrocytic stages to primaquine when administered in different dose schedules to rhesus monkeys infected with P.cynomolgi sporozoites.

The sensitivity and protective potential of primaquine has been based on the assessment of protection accorded by single dose, two doses and three doses of primaquine (base) administered in each instance on different days of incubation period (Table 11) Primaquine at 1.78mg/kg in 3 dose schedule (=5.34 mg/kg total course dose) afforded protection in 4/4 monkeys treated on day -1, 0, +1 and in 2/2 monkeys treated on day +1, +2, +3. None of the monkeys treated on days +3, +4, +5 (0/2 monkeys) or on days +5, +6, +7 (0/2 monkeys) post sporozoite inoculation were protected. Similar results were obtained using primaquine at 2.67mg/kg in 2 dose schedule (=5.34mg/kg total course dose) where 2/2 monkeys were protected when treated on day = and +1. No protection was obtained after treatment on day +3 and +4 (0/2 monkeys) or on days +6 and +7 (0/2 monkeys).

Single dose primaquine administration at 5.34mg/kg was curative in 2/2 monkeys treated on day 0, 2/2 monkeys treated on day +1 and in 1 out of 2 monkeys treated on day +2. Single dose treatment on day +3 protected 1 out of 8 monkeys and produced significant delay in onset of patency in another monkey. Single dose treatment was not protective in 2 monkeys each treated either on day -1 or on day +6.

Further, studies with single dose primaquine showed that treatent at 2.67mg/kg (= half the total curative course dose) afforded protection in 1 out of 2 monkeys given drug on day 0, while none of the 2 monkeys treated on day +1 and none of 3 monkeys treated on day +3 was protected.

Primaquine administration at 10.68mg/kg (= twice the total curative course dose) protected 3/3 monkeys treated on day +3 and 1 of the 2 monkeys treated on day +6, while the other unprotected monkey showed significantly delayed patency.

These results support the suitability of 3-day regimen model (-1, 0, +1 day) for development of new causal prophylactic agents.

E. NEW ANTIMALARIAL TEST-SYSTEMS:

The following additional antimalarial screen has been standardized at CDRI.

(a) Rhesus Gametocytocidal/Sporontocidal Test:

No standardized technique for determining gametocytocidal activity/sporontocidal action of new compound against vivax type of simian malaria namely P.cynomolgi B has been developed so far. Primaquine is the only standard gametocytocidal drug available and there is urgent need to screen the primaquine analogues which have shown high anti-relapse activities and establish their gametocytocidal activity. Under the continuing programme of CDRI-WRAIR collaborative project, it is poroposed to initiate studies on the development of this test using P.cynomolgi - A.stephensi model. The capability of producing infectivity in the mosquitoes, recording of gametocytaemia, oocyst count, etc. are routinely carried out under the existing project. The experience and expertise available at WRAIR and CDRI will be used to develop new gametocytocidal agents.

Gametocytocidal test:

Primaquine was used as a standard reference drug to establish the model ofr gametocytocidal activity. Rhesus monkeys infected with P.cynomolgi and carrying gametocytes as shown by Giemsa stained blood film were first used for control (pre-treatment) feeding at -1 hr. before primaquine treatment. This group comprising of seven monkeys was then treated with single oral dose of primaquine diphosphate (3.16mg/kg primaquine base). New healthy batches of mosquitoes were again allowed to feed on the primaquine treated monkeys at 24 and 48 hr.

after treatment. The data on oocyst count in control feeding and after drug feeding were statistically analysed (Table 12).

Sporontocidal test:

In the test system developed for sporontocidal screening the reference drug primaquine was not fed directly to the infected vector but the drug was given orally to healthy rhesus monkey and 5 hrs. after drug administration 3-5 days old infected A.stephensi were fed on this drug treated monkey. The dose of primaquine administered in this test was 10mg/ base/kg (single dose) by oral route. The oocyst count on the gut of individual mosquitoes was recorded on day 8 post-infection (Table 13).

BLOOD SCHIZONTOCIDAL TEST:

a) CHLOROQUINE DOSE:

i) P.cynomolgi infection:

Tests using trophozoite induced infection of P.cynomolgi B in rhesus monkeys have shown that chloroquine at 3.00mg/kg dose x7 days continues to exert complete blood schizontocidal activity as no recrudescence was observed in the treated monkeys.

ii) P.fragile infection:

Another test model using P.fragile infection in rhesus monkey has been standardized for evaluating blood schizontocidal activity of new compounds. In this model, chloroquine at a dose of 7.5mg/kgx3doses has been determined as the curative dose.

b) EVALUATION OF WR 238605 FOR BLOOD SCHIZONTOCIDAL ACTIVITY:

Compound WR 238605 had been selected under the previous WRAIR-CDRI collaborative project as the candidate antimalarial to undergo Phase I clinical trials for development as an alternative tissue schizontocidal drug. In the present programme, this compound was evaluated for its blood schizontocidal activity against P.cynomolgi B and P.fragile which are considered as the biological counterparts to human parasites P.vivax and P. falciparum respectively.

i) Activity against P.cynomolgi B:

The activity was tested at 0.316mg/kg, 1.00mg/kg and 3.16mg/kg doses. Results showed that three monkeys at 0.316mg/kg dose recrudesced on days 7, 18 and 23 while four monkeys at 1.00mg/kg and three monkeys at 3.16mg/kg were cured. The

monkeys which became patent at lower dose of 0.316mg/kg were treated at higher dose with WR 238605 and were cured (Table 14).

In the 2nd experiment 4 monkeys were treated at 1.00mg/kg dose. One monkey recrudesced on day 26 while remaining three were cured (Table 15).

ii) **Activity against P.fragile**:

Efficacy tests showed that in the 1st experiment two monkeys treated at 0.316mg/kg recrudesced on days 16 and 19. Three monkeys at 1.00mg/kg and three monkeys at 3.16mg/kg were cured after 7 day treatment. Two monkeys which failed at 0.316mg/kg dose were treated again at higher dose of 1.00mg/kg and both were cured (Table 16).

In the 2nd experiment two monkeys at 0.316mg/kg dose recrudesced on days 24 and 28. Three out of 4 monkeys at 1.00 were cured while fourth monkey was positive on day 36. This monkey when treated at 3.16mg/kg dose was protected (Table-17).

MAJOR CONCLUSIONS

1. Antimalarial test screens for the evaluation of potential compounds for anti-relapse activity, causal prophylactic activity and blood schizontocidal activity have been maintained in the operational state at CDRI.

2. The P.cynomolgi B strain has been maintained for 72 successive cyclic passages, since Nov. 1982. The parasite passed through serial passages 63-72 during the tenure of present assignment and the mean duration for each passage was 44.89±8.16 days.

3. The causal prophylactic dose of primaquine has been revalidated and the 1.78mg/kgx3day dose has been found curative. There has been no escalation of the primaquine prophylactic dose during the last 8 years.

4. Six new compounds have been evaluated for causal prophylactic activity. One compound WR 268404 has shown primaquine index of 5.63 while the other 5 compounds had P.I.=1.78. Whether any of these compounds can be followed for pre-clinical development, will be decided after methemoglobin toxicity data on these compounds is obtained.

5. Test models for evaluation of potential compounds for gametocytocidal and sporontocidal actvity using P.cynomolgi B infection has been standardized. It is proposed to evaluate compound WR 238605 (undergoing Phase I clinical trials at WRAIR) for gametocytocidal and sporontocidal activities.

6. Compound WR 238605 has shown significant blood schizontocidal activity against two simian malaria parasites. This data will add to the antimalarial spectrum of this compound which is being developed as a potential tissue schizontocidal agent.

Causal prophylactic activity of primaquine in 3 day treatment schedule against sporozoite induced infections of P.cynomolgi in rhesus monkey.

Daily dose mg/kg base (x3day)	No. of monkeys protected/treated	Days delay in onset of patency	Percent cure
0.316	0/6	0,2,3,5,6,7	Nil
0.62	0/5	5,6,6,6,7	Nil
1.00	9/16	7,7,9,10,11,16,24	56.3%
1.30	2/2	Cured	100%
1.78	26/26	Cured	100%
3.16	11/11	Cured	100%
10.00	4/4	Cured	100%

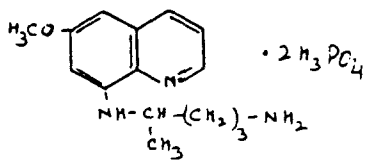
Table-2

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPOROZOITE INDUCED TEST

COMPD: WR 2975

BN: Sigma product

DATE REC'D: Oct. 1982



QUANTITY: 50 gm.

VEHICLE: Methyl cellulose

Mol. Wt.= 457

ROUTE: Oral

Base= 259

PROPHYLACTIC TEST (x3day)

DOSE mg/kg(base)	SERIAL SPOROZOITE PASSAGE NO	MONKEY NO.	RESULT
1.78	XV	2798	Cured
1.78	XV	2799	Cured
1.78	XVI	2815	Cured
1.78	XXI	3220	Cured
1.78	XXII	3334	Cured
1.78	XXIII	3363	Cured
1.78	XXIII	3364	Cured
1.78	XXIV	3475	Cured
1.78	XXIV	3476	Cured
1.78	XXV	3502	Cured
1.78	XXVIII	3683	Cured
1.78	XXVIII	3753	Cured
1.78	XXIX	3813	Cured
1.78	XXXI	3993	Cured
1.78	XXXI	3997	Cured
1.78	XXXIV	4353	Cured
1.78	XXXX	4675	Cured
1.78	XXXX	4678	Cured
1.78	XXXXI	4730	Cured

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPOROZOITE INDUCED TEST

COMPD: Primaquine

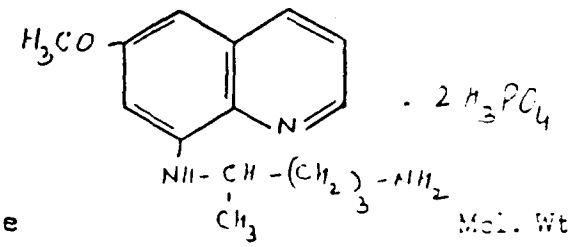
BN: Sigma Product

DATE REC'D: Oct. 1988

QUANTITY: 50 gm

VEHICLE: Methyl cellulose

ROUTE: Oral



Mol. Wt.= 455

Base= 259

PROPHYLACTIC TEST (x3day) (Sp. passage no. 65)

DOSE mg/kg(base)	MONKEY NO.	RESULT
1.00	6228	Cured
1.00	6229	Patency on day 36
1.00	6230	Patency on day 36
1.78	6235	Cured
1.78	6238	Cured
1.78	6242	Cured
3.16	6227	Cured
3.16	6232	Cured
3.16	6233	Cured
Vehicle control	6241	Patency on day 8

Table-4

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPOROZOITE INDUCED TEST

COMPD: WR 268658

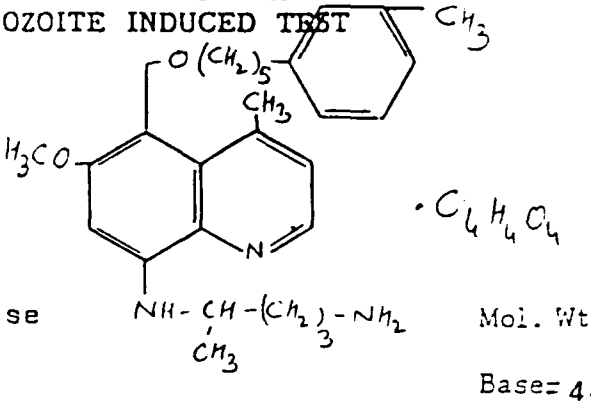
BN: BM 03705

DATE REC'D: June, 1990

QUANTITY: 500 mg

VEHICLE: Methyl cellulose

ROUTE: Oral



Mol. Wt.= 565

Base= 449

PROPHYLACTIC TEST (x3day) (Sp. passage no. 67)

DOSE mg/kg(base)	MONKEY NO.	RESULT
0.0316	6387	Patent day 8
0.0316	6390	Patent day 8
0.1	6382	Patent day 11
0.1	6388	Patent day 10
0.316	6384	Patent day 23
0.316	6386	Patent day 11
Vehicle control:	6385	Patent day 8

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPOROZOITE INDUCED TEST

COMPD: WR 268658
(IInd experiment)

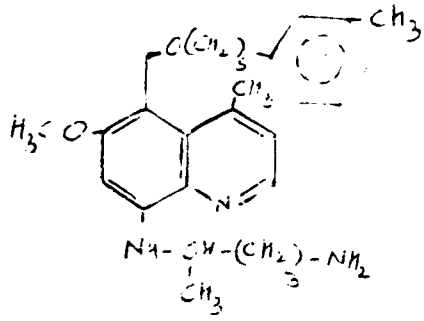
BN: BM 03705

DATE REC'D: June, 1990

QUANTITY: 500 mg

VEHICLE: Methyl cellulose

ROUTE: Oral



Mol. Wt.= 565

Base= 449

PROPHYLACTIC TEST (x3day) (sp. passage No. 68)

DOSE mg/kg(base)	MONKEY NO.	RESULT
1.00	6405	Cured
1.00	6406	Cured
Vehicle control	6398	Patent day 8

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPOROZOITE INDUCED TEST

COMPD: WR 268448

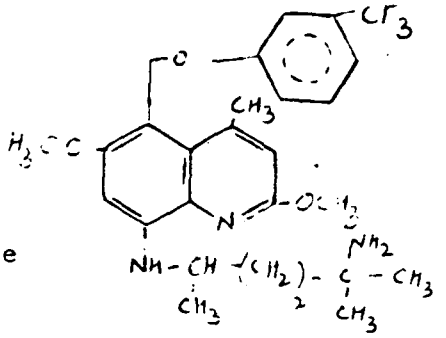
BN: BM 01194

DATE REC'D: Oct. 1989

QUANTITY: 1500 mg

VEHICLE: Methyl cellulose

ROUTE: Oral



Mol. Wt.= 590

Base= 491

PROPHYLACTIC TEST (x3day) (Sp. passage no. 68)

DOSE mg/kg(base)	MONKEY NO.	RESULT
0.10	6402	Patent day 10
0.10	6407	Patent day 10
0.316	6400	Patent day 13
0.316	6404	Patent day 23
1.00	6401	Cured
1.00	6403	Cured
Vehicle control	6398	Patent day 8

Table-6

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPOROZOITE INDUCED TEST

COMPD: WR 268441

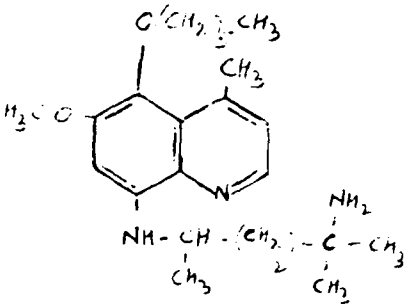
BN: BM 01103

DATE REC'D: June, 1990

QUANTITY: 1500 mg

VEHICLE: Methyl cellulose

ROUTE: Oral



Mol. Wt.= 539

Base= 401

PROPHYLACTIC TEST (x3day) (sp. passage 68)

DOSE mg/kg(base)	MONKEY NO.	RESULT
0.10	6437	Patent day 17
0.10	6438	Patent day 12
0.316	6435	Cured
0.316	6436	Patent day 29
1.0	6429	Cured
1.0	6430	Cured
Vehicle control	6440	Patent day 9

Table-7

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPORozoite INDUCED TEST

COMPD: WR 268648

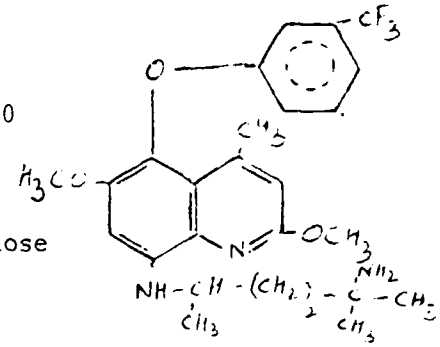
BN: BM 03590

DATE REC'D: June, 1990

QUANTITY: 500 mg

VEHICLE: Methyl cellulose

ROUTE: Oral



Mol. Wt.= 579

Base= 463

PROPHYLACTIC TEST (x3day) (Sp. passage no.68)

DOSE mg/kg(base)	MONKEY NO.	RESULT
0.10	6459	Patent day 12
0.10	6473	Patent day 14
0.316	6458	Patent day 21
0.316	6472	Patent day 26
1.00	6465	Cured
1.00	6470	Cured
Vehicle control	6464	Patent day 8

Table-5

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPOROZOITE INDUCED TEST

COMPD: WR 268404

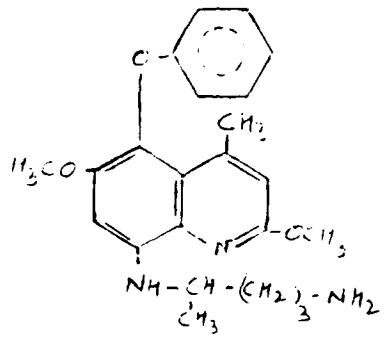
BN: BM 00722

DATE REC'D: June, 1990

QUANTITY: 1500 mg

VEHICLE: Methyl cellulose

ROUTE: Oral



Mol. Wt.= 511

Base= 395

PROPHYLACTIC TEST (x3day) (Sp. Passage No. 68)

DOSE mg/kg(base)	MONKEY NO.	RESULT
0.10	6456	Patent day 12
0.10	6457	Patent day 15
0.316	6471	Cured
0.316	6474	Cured
1.0	6462	Cured
1.0	6463	Cured
Vehicle control	6464	Patent day 8

CDRI PRIMATE ANTIMALARIAL STUDY
PLASMODIUM CYNOMOLGI B RHESUS MONKEY
SPOROZOITE INDUCED TEST

COMPD: WR 268499

BN: BM 01667

DATE REC'D: June, 1990

QUANTITY: 1500 mg

VEHICLE: Methyl cellulose Mol. Wt.= 557

ROUTE: Oral Base=441

PROPHYLACTIC TEST (x3day) (Sp. passage no. 69)

DOSE mg/kg(base)	MONKEY NO.	RESULT
0.10	6585	Patent day 22
0.10	6587	Patent day 15
0.316	6581	Cured
0.316	6584	Patent day 26
1.00	6582	Cured
1.00	6586	Cured
Vehicle control	6580	Patent day 8

Table: Serial cyclic passage of sporozoite induced P.cynomolgi B in rhesus monkeys since February, 1990.

Sporozoite Passage No.	Date of inoculation	Monkey No.	Sporozoite inoculum (i.v.)	Day of patency
63	25.1.90	6118	1.06×10^6	9
64	9.3.90	6177	1.48×10^5	10
65	17.4.90	6241	1.18×10^6	8
66	28.5.90	6302	1.21×10^6	8
67	3.7.90	6385	0.98×10^6	8
68	1.8.90	6398	0.75×10^6	8
	20.8.90	6440	0.66×10^6	9
	31.8.90	6464	0.82×10^6	8
69	24.10.90	6580	1.06×10^6	8
70	6.12.90	6523	1.14×10^6	8
71	10.1.91	6586	0.91×10^6	8
72	5.3.91	6775	1.30×10^6	8

Table 11: Causal prophylactic activity of single and multiple dose regimens of primaquine against P.cynomolgi B in rhesus monkeys

Daily dose x days (mg/kg)	Treatment Day *	Total course dose mg/kg	Cure rate (protected)/(treated)	Patency on day
SINGLE DOSE				
2.67 x 1	0	2.67	1/2	20
2.67 x 1	1	2.67	0/2	14,15
2.67 x 1	3	2.67	0/3	14,16,24
5.34 x 1	-1	5.34	0/2	11,11
5.34 x 1	0	5.34	2/2	
5.34 x 1	1	5.34	2/2	
5.34 x 1	2	5.34	1/2	20
5.34 x 1	3	5.34	1/8	14,21,21, 25,26,30&3
5.34 x 1	6	5.34	0/2	14,21
10.68 x 1	3	10.68	3/3	
10.68 x 1	6	10.68	1/2	43
Two Doses				
2.67 x 2	0,1	5.34	2/2	
2.67 x 2	3,4	5.34	0/2	28, 37
2.67 x 2	6,7	5.34	0/2	16, 43
Three Doses				
1.78 x 3	-1,0,1	5.34	4/4	
1.78 x 3	1,2,3	5.34	2/2	
1.78 x 3	3,4,5	5.34	0/2	24, 29
1.78 x 3	5,6,7	5.34	0/2	26,39

* Day 0 = Day of sporozoite inoculation

able- : Gametocytocidal activity of primaquine diphosphate as shown by oocyst counts on gut of A.stephensi fe on infected monkey.

onkey No. Infection)	Mosquito feeding	Asexual parasit-aemia (%)	Gametocy-taemia (%)	Primaquine dose	Mosquito dissection No. infected / No. examined	Infectivity rate	Oocyst count (Mean±SD)
	Pre-treatment						
. 4767 (Blood induced)	1. at -25h	0.7	0.05	-	8/8	100%	89.62±41.45
	2. at -1h	.08%	.001%	-	5/6	83.33%	10±6
	Post-treatment						
	3. at 24 h	.02%	.001%	1mg/kgx1	1/33	3.33%	1±1
	Pre-treatment						
. 4769 (Blood induced)	1. at -25h	0.8%	0.1%	-	6/11	54.54%	16.5±14.13
	2. at -1h	0.6%	0.07%	-	4/7	57.14%	67.5±42.13
	Post-treatment						
	3. at 24h	0.12%	.001%	3.16mg/kgx1	Nil/21	Nil	Nil
	Pre-treatment						
3. 4909 (Sporo-zoite induced)	1. at -1h	1.5%	.25%	-	13/16	81.25%	16.15±14.62
	Post-treatment						
	2. at 24h	.2%	.09%	3.16mg/kgx1	Nil/50	Nil	Nil
	3. at 48h	.07%	.01%	-	Nil/25	Nil	Nil

ntinued Table- :

	Pre-treatment						
4910 (Sporo-zoite induced)	1. at -1h	2.0%	.25%	-	9/9	100%	66.3332.49
	Post-treatment						
	2. at 24h	.12%	.004%	3.16mg/kg x1	Nil/56	Nil	Nil
	3. at 48h	.1%	0.001%	-	Nil/30	Nil	Nil
	Pre-treatment						
4940 (Sporo-zoite induced)	1. at -1h	.15%	0.02%	-	7/19	36.84%	3.71±2.21
	Post-treatment						
	2. at 24h	0.05%	Nil	3.16mg/kg x1	Nil/24	Nil	Nil
	Pre-treatment						
4941 (Sporo-zoite induced)	1. at -1h	.5%	0.04%	-	18/31	58.06%	3.55±2.45
	Post-treatment						
	2. at 24h	.53%	0.01%	3.16mg/kg x1	Nil/25	Nil	Nil
	Pre-treatment						
4907 (Sporo-zoite induced)	1. at -1h	.2%	0.05%	-	6/16	37.5%	3.5±2.42
	Post-treatment						
	2. at 24h	0.05%	0.005%	3.16mg/kg x1	Nil/25	Nil	Nil

Table-12 (Contd.)

Table- Sporontocidal test with primaquine administered to healthy monkey, on which infected (*P.cynomolgi* B) mosquitoes (*A.stephensi*) were allowed to feed.

Age of mosquito infection	Primaquine treatment	Mosquito dissection No. infected/No. examined	Infectivty (%)	Ooocyst count (Mean±SD)
1. 3 day	10mg/kg at -5h	22/26	84.62	45.32±23.19
Control	Untreated	20/24	83.33	53.6±24.29
2. 4 day	10mg/kg at -5h	20/25	80.00	84.75±29.84
Control	Untreated	22/27	81.48	101.36±29.08
3. 5 day	10mg/kg at -5h	27/33	81.82	22.85±9.65
Control	Untreated	28/35	80.00	26.93±12.02

Table-13

Note : 1. Drug was administered to healthy monkey 5 hours before mosquito feeding.

2. Results of feeding with 3.16 mg base/kg primaquine were similar to above.

CDRI PRIMATE ANTIMALARIAL STUDY

PLASMODIUM CYNOMOLGI - RHESUS MONKEY

COMPD: WR 238605 (Expt. I)

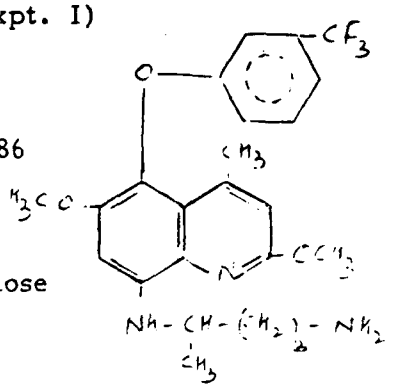
BN: BK 73252

DATE REC'D: Feb. 1986

QUANTITY: 3gm

VEHICLE: Methyl cellulose

ROUTE Oral



Mol. Wt.= 581

Base= 463

DOSE mg/kg(base)	MONKEY NO.	RESULT
	BLOOD SCHIZONTOCIDAL TEST	
0.316	6547	Recrudescence day 13
0.316	6548	Recrudescence day 18
0.316	6554	Recrudescence day 7
1.00	6551	Cured
1.00	6553	Cured
1.00	6556	Cured
1.00	6548*	Cured
3.16	6549	Cured
3.16	6550	Cured
3.16	6555	Cured
3.16	6547*	Cured
3.16	6554*	Cured

*Monkeys treated at higher dose after recrudescence.

CDRI PRIMATE ANTIMALARIAL STUDY

PLASMODIUM CYNOMOLGI - RHESUS MONKEY

COMPD: WR 238605 (IInd Expt.)

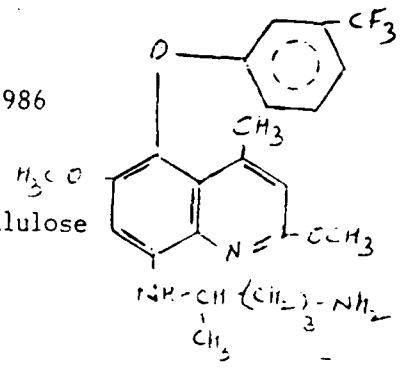
BN: BK 73252

DATE REC'D: Feb. 1986

QUANTITY: 3gm

VEHICLE: Methyl cellulose

ROUTE Oral



Mol. Wt.= 581

Base=463

DOSE mg/kg(base)	MONKEY NO.	RESULT
BLOOD SCHIZONTOCIDAL TEST		
1.00	6640	Cured
1.00	6641	Cured
1.00	6642	Recrudescence day 26
1.00	6643	Cured
3.16	6642*	Cured

*Monkey treated at higher dose after recrudescence.

Table-16

CDRI PRIMATE ANTIMALARIAL STUDY

PLASMODIUM FRAGILE - RHESUS MONKEY

COMPD: WR 238605 (Expt.1)

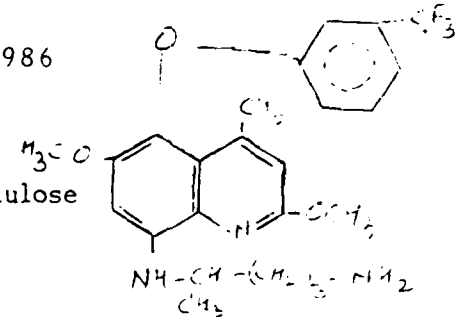
BN: BK 73252

DATE REC'D: Feb. 1986

QUANTITY: 3gm

VEHICLE: Methyl cellulose

ROUTE Oral



Mol. Wt.= 581

Base= 463

DOSE mg/kg(base)	MONKEY NO.	RESULT
BLOOD SCHIZONTOCIDAL TEST		
0.316	6559	Recrudescence day 19
0.316	6564	Recrudescence day 16
1.00	6557	Cured
1.00	6558	Cured
1.00	6560	Cured
1.00	6559*	Cured
1.00	6564*	Cured
3.16	6561	Cured
3.16	6562	Cured
3.16	6565	Cured

*Monkeys treated at higher dose after recrudescence

CDRI PRIMATE ANTIMALARIAL STUDY

PLASMODIUM FRAGILE - RHESUS MONKEY

COMPD: WR 238605 Expt. 2)

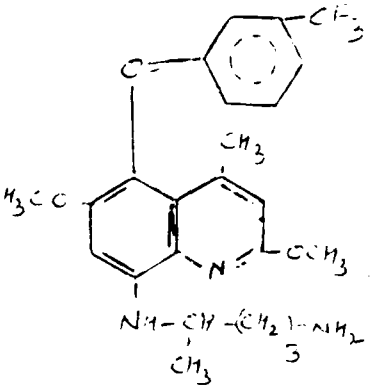
BN: BK 73252

DATE REC'D: Feb. 1986

QUANTITY: 3gm

VEHICLE: Methyl cellulose

ROUTE Oral



Mol. Wt.= 581

Base= 463

BLOOD SCHIZONTOCIDAL TEST

DOSE mg/kg(base)	MONKEY NO.	RESULT
0.316	6646	Recrudescence day 28
0.316	6657	Recrudescence day 24
1.00	6644	Recrudescence day 2[illegible]
1.00	6645	Cured
1.00	6647	Cured
1.00	6657	Cured
1.00	6646*	Cured
1.00	6657*	Cured
3.16	6644*	Cured

*Monkey treated at higher dose after recrudescence